FURTHER STUDIES OF GENE-CONTROL SYSTEMS IN MAIZE

Barbara McClintock

Continued study of the *Spm* system of control of gene action in maize has produced additional evidence of the system's versatility. A control system of this type is capable of regulating the action of many genes during development. In view of the large number of genes present in the nuclei of higher organisms, and the need for coordination of their action during development, it is reasonable to assume that evolutionary processes would have initiated superregulatory mechanisms to accomplish that end. The *Spm* system serves as a model of the mode of operation of one type of superregulatory mechanism. Such a system can activate or inactivate particular genes in some cells early in development, and activate or inactivate other genes later in development. It can turn on the action of some genes at the same time that it turns off the action of others. It can adjust the level of activity of a particular gene in different parts of an organism. Some evidence to support these statements has been presented in previous *Year Books* and other publications, and additional evidence will be reported here.

At the time they were first detected, controlling elements were distinguishable from genes because, unlike genes, they could be transposed from one location to another in the chromosome complement. Thus the operator element of a regulatory system may be inserted at the locus of a gene, whose action is then subject to control by the system to which the operator element belongs. Because the detected controlling elements of a system may reside at different locations in the chromosome complement, they have been likened to episomes in bacteria. This comparison is justified, since it is known that the activity of bacterial genes may be subject to control by episomes. In maize, however, the operator element of either the *Ac* (Activator) or the *Spm* (Suppressor-mutator) system may become integrated into a chromosome in such a way that it no longer undergoes transposition. In its fixed position it continues to respond to signals from the regulator, and its responses are made evident by changes in action of the gene with which the integrated operator element is associated. At present, there is no direct evidence for or against episomal origin of the components of known gene-control systems in maize. It is conceivable, nevertheless, that some superregulatory mechanisms in higher organisms may have originated through incorporation and adaptation of the gene-control components of episomes.

The *Ac* and *Spm* gene-control systems are not considered to play a vital role in coordinating gene action during development of the maize plant, at least as far

as the known states of the component elements are concerned. If they did play such a role, it is doubtful that they could have been analyzed so readily; for then the selected mutants of the regulator elements, which have provided much evidence about the modes of operation of the systems, would have so altered coordination as to affect adversely the growth of plant or kernel. Adverse effects have not been noted. Thus, the controlling elements of the examined systems may represent foreign, nonessential, episome-like components that have been integrated into the maize genome; or, on the other hand, they may be true chromosomal components of present-day maize, whatever their evolutionary origins and histories may have been. Some evidence supporting the second consideration will be reviewed briefly.

My study of gene-control systems in maize was initiated with an experiment conducted some years ago, which utilized the chromosome type of breakage-fusion-bridge cycle. The unexpected results of this experiment, reported at the time, should again be emphasized here. In the self-pollinated progeny of a number of plants that had commenced development with a chromosome pair undergoing the breakage-fusion-bridge cycle, it was startling to observe that many unrelated genes, whose action had previously been normal with respect to expression in plant tissues, were now exhibiting marked differences in activity in different parts of plants. The patterns of change in the somatic tissues, regardless of the cellular function with which a particular modified gene was concerned (that is, plastid development, chlorophyll synthesis, anthocyanin synthesis, or capacity of the cell for growth or division), reflected some mechanism of control of action of these genes during development.

Studies were then begun to examine these gene-control mechanisms. Later, when the components of a control system were recognized, it was possible to reconsider the basic question posed by the results of the initial experiment: Why were control systems so suddenly revealed and at so many different gene loci? It was concluded that regulatory elements, distinct from the genes, must have been present in the nuclei of the maize plants before the breakage-fusion-bridge-cycle experiment was conducted, and that this cycle, in some yet unknown manner, had induced modifications in the pre-existing elements. The evidence was so compelling that it was later decided to test the conclusion. If it was correct, the breakage-fusion-bridge cycle should be able to induce a known type of regulatory element from some unknown element in a nucleus whose ancestor nuclei, in their past history, through many plant generations, had never given evidence of its presence. The experiment, reported in *Year Book 50*, was successful. There was no doubt that *Dt*-type regulation could be induced, independently, in a number of different nuclei where the cycle was in effect. It was also evident that each induction was the consequence of a single event involving some component in an individual cell. After induction, the regulatory activity of the induced element was registered in the descendent cells.

Additional evidence for considering that the examined types of controlling elements are derived from components normally present in maize chromosomes comes from studies of induction of change in action of a presumed "wild-type" gene by particular alleles of the gene. This induction effect is termed "paramutation" by R. A. Brink in studies of alleles of the *R* gene, located in chromosome 10, and is termed "conversion" by E. H. Coe in studies of alleles of the *B* gene, located in chromosome 2. With regard to either *R* or *B*, the initially recognized allele that induced change in action of a presumed normal allele would have been placed in the "mutable gene" category had its behavior been analyzed in earlier years. Indeed, genes under the control of the *Ac*, *Spm*, and *Dt* systems were at first referred to as mutable genes, since they exhibited phenotypes to which this term had been applied in many early genetic

investigations. It seems most likely that induction of change in gene action of "wild-type" alleles, and the subsequent capacity of some of the changed alleles to induce similar changes, resides in controlling elements normally associated with the genes.

The resemblance between effects brought about by the inducing alleles, described above, and those produced by elements of known control systems is further illustrated in studies conducted by Dr. Brink and his students with the *R* alleles. The initially recognized inducing allele, R^{st}, gives rise to a distinctive pattern of pigmented areas in a nonpigmented background in the aleurone layer of the kernel. The frequency of occurrence of change from inactive to active expression of the *R* gene in the cells of the endosperm during its development may be determined readily by examining the numbers of such pigmented areas in the kernel. There is a dominant modifier that markedly increases the frequency of occurrence of these events without altering the time during development when they take place. Initially, this modifier was placed approximately six crossover units distal to the locus of R^{st}. Its effect resembles in detail that of a dominant modifier element in the *Spm* system, reported in *Year Book 57*, and like that element it undergoes transposition. Thus, accumulating evidence obtained in studies of the *R* and *B* alleles now makes it reasonable to consider that "paramutation" and "conversion" reflect alterations induced by and in components of gene-control systems normally present in maize. It would be instructive to determine, therefore, whether or not the individual components of these systems could be identified through transposition and insertion elsewhere in the chromosome complement.

Modified States of a_1^{m-2}

In *Year Book 61* the mode of control by the *Spm* system of gene action at a_1^{m-2} was reviewed. As was stated there, the regulator element, *Spm*, resides close to the locus of the A_1 gene. When this element is in an active phase, the A_1 gene is active, but during an inactive phase of the element gene action is inhibited unless an active *Spm* element is present elsewhere in the chromosome complement. When an active *Spm* is present, release of gene action from the control of the *Spm* system occurs in some somatic cells, and is accompanied by the production of mutants of the A_1 locus, the classes of which were described last year. When the *Spm* element associated with the a_1^{m-2} locus is inactive, and no additional active *Spm* is present in the nuclei of plant or kernel, such mutants are not produced.

In this study of a_1^{m-2}, an occasional kernel on a testcross ear exhibited an atypical phenotype with respect to expression of the A_1 gene. Plants were grown from some of these kernels, and each was subjected to testcrosses in order to explore the cause of the modified gene expression. It could be determined by this means that the atypical phenotypes arose through modification of either the regulator element, *Spm*, or the operator component residing at the A_1 locus.

Modified states of Spm at the a_1^{m-2} *locus.* There are several readily recognized types of modification of the *Spm* element. One type changes its phase of activity; another type alters the time of its transposition during development; a third markedly alters its capacity to effect responses of the operator element that result in release of gene action from the control of the *Spm* system. The third type of modification, discovered initially in studies of a_1^{m-1} and a_2^{m-1}, was designated Spm^w and was described in *Year Book 56*. With a_1^{m-1} and the class I states of a_2^{m-1}, the inhibitory effect of Spm^w on gene action at either of these loci is pronounced, but its capacity to induce responses of their operator elements that release the genes from *Spm* control is very much weakened. Few mutants of this type are produced.

The type of control of A_1 gene action

when an *Spm*^{w} is present at the a_1^{m-2} locus is that expected. The gene is active, since the inhibitory effect of *Spm* on gene action at a_1^{m-2} is the reverse of that on gene action at a_1^{m-1} and a_2^{m-1}. In the kernels, pigment of medium intensity is distributed over the aleurone layer. The weakened capacity of *Spm*^{w} to induce modification of gene action is expressed in the kernel either by an absence of mutant areas or by the presence of one or several very small areas showing a mutant phenotype. Thus, *Spm*^{w} differs from standard *Spm* (which in this account will be designated *Spm*^{s}) in capacity to effect mutation-inducing responses of the operator element of the system.

Two isolates of a_1^{m-2} in which the associated *Spm*^{s} element had mutated to *Spm*^{w} were examined this past year. The *Spm*^{w} mutants differ in frequency of reversion to *Spm*^{s}, one reverting frequently and the other rarely. Previous studies of *Spm*^{w} mutants, conducted with plants having either a_1^{m-1} or a_2^{m-1}, had shown that, when *Spm*^{w} and *Spm*^{s} are both present in the nuclei of a plant or kernel, *Spm*^{s} exerts a dominant effect. *Spm*^{w} is not altered by the association, however, but can be recovered quite unchanged in the progeny of plants having both the *Spm* elements. Each of the isolates of a_1^{m-2} with *Spm*^{w} located close to it responds in this same manner to *Spm*^{s} located elsewhere in the chromosome complement, and the response is similar to that seen when *Spm*^{s} resides at the locus of a_1^{m-2}. Moreover, tests conducted with one of these isolates have indicated that *Spm*^{w} is recovered unaltered in the progeny of plants in which an independently located *Spm*^{s} element is also present.

Altered states of the operator element at a_1^{m-2}. Kernels exhibiting atypical phenotypes were selected with the intent of finding among them examples in which the regulator element, *Spm*, but not the operator element had been removed from the locus of a_1^{m-2}. Tests were initiated with plants derived from 7 selected kernels, and continued with their progeny. It was learned that the modified phenotype in 4 of these kernels could be ascribed neither to an event that had removed *Spm* from the locus of a_1^{m-2} nor to change in *Spm* action. These 4 modified states of a_1^{m-2} differ from the original state mainly in the frequency of occurrence of each of the types of A_1 gene expression that arise in conjunction with release of the gene from control of the *Spm* system. The remaining 3 altered states show no evidence of the presence of *Spm* at the modified a_1^{m-2} locus. Each, however, responds in its own way to an active *Spm* element located elsewhere in the chromosome complement. Two of these states promise to provide new evidence about kinds of control of gene expression during development.

One of them illustrates the manner in which anthocyanin pigment of different intensities may be produced in individual cells of a tissue. Such differences may be due to "cross-feeding" between adjacent cells that, individually, either cannot synthesize pigment or can synthesize very little. The phenomenon is suggested by the distribution of pigmented cells in kernels having one of the two mentioned states of a_1^{m-2} and an active *Spm* element. These kernels have pigmented areas in a colorless or nearly colorless background. Some areas are not solidly pigmented, but are bounded by a band of pigmented cells. The intensity of pigment in the cells composing these ring-shaped bands is not uniform. Many of the bands have an inner row of deeply pigmented cells. The cells on both sides of this inner row exhibit a decreasing gradient of intensity of pigment, fading gradually into colorless or nearly colorless cells. The origin of the ring-shaped bands, in which some cells exhibit pigment intensities approaching that produced by the normal A_1 gene, may be explained by cross-feeding between adjacent cells that differ genotypically. The shapes of the pigmented bands indicate that the cells enclosed by each band are descendants of one in

which the genotype was modified, and the modifications appear to have been controlled by the *Spm* system.

The second of these two modified states of a_1^{m-2} was mentioned in *Year Book 61* during a discussion of a_1^{m-2} that considered potential isolates having an operator element of the *Spm* system but no *Spm* regulator element associated with the locus. This state was detected, initially, in a kernel that exhibited an atypical phenotype characterized by some lightly pigmented areas in a colorless background. The intensity of anthocyanin pigmentation differed among the cells of an area: some were more intensely pigmented than others, and some appeared to have no pigment, but none was deeply pigmented. As was reported last year, there was no evidence of the presence of *Spm* in the plant derived from this kernel.

The results obtained in initial tests conducted with this plant and in more extensive tests of its progeny will be summarized here. It was learned that the phenotype described above appears in kernels in subsequent generations as long as the altered state of a_1^{m-2} has not passed through a plant generation in which an active *Spm* element is also present in the nuclei. When an active *Spm* is introduced by appropriate crosses, the phenotype of the kernels that have received the altered a_1^{m-2} locus from one parent and an active *Spm* from the other parent, and also the phenotype of the plants derived from these kernels, is similar to that produced by the original state of a_1^{m-2}. When testcrosses were conducted, however, with the plants that had received a newly introduced *Spm*, some of the kernels on the resulting ears exhibited a decidedly modified phenotype, which appeared only among kernels that had received the altered state of a_1^{m-2} but no *Spm*. These kernels had mottled areas, within which many of the cells were intensely pigmented. The presence of intense pigmentation in the mottled areas allowed recognition of confluence of these areas in a manner that gave rise to a clearly defined pattern of pigment distribution over the aleurone layer. Microscopic examination of the kernels showed that the distribution and intensity of pigment in individual cells within an area very much resembled those produced by the kind of cross-feeding between adjacent cells described above.

Among the kernels on these ears that had a_1^{m-2} but no *Spm*, a wide range in expression of maximum pigment intensities was noted, although within an individual kernel all the mottled areas showed the same range of intensity of cell pigmentation. Among the different kernels the maximum intensity ranged from very dark to rather faint. Kernels with strikingly enhanced pigmentation in the mottled areas appeared on ears produced by crosses conducted with each of 11 tested plants that commenced development with a newly introduced *Spm*. They did not appear on ears produced by similar crosses conducted with 26 sister plants that had not received *Spm*. Therefore it is suspected that *Spm* induced, in many cells of the germ line, a particular type of modification of the operator element of the altered state of a_1^{m-2}. This change enhanced the capacity of the A_1 gene to contribute to pigment formation in cells of a mottled area after *Spm* had been removed from the nucleus in the meiotic process. In some respects this phenomenon resembles the paramutagenic effect described earlier.

Study of a_1^{m-2} has added to our appreciation of gene-control mechanisms in that it suggests cooperation between two or more regulatory mechanisms. With the original state, activation and inactivation of the A_1 gene are controlled by *Spm*. Another regulatory mechanism must operate, however, when the gene is active. This fact was revealed early in the study of a_1^{m-2}, by the restricted distribution of anthocyanin in plant tissues described in *Year Book 61*. It is also strikingly revealed by the very distinctive pattern of arrangement of mottled areas

in the kernels just described. It is again manifested by the distribution of pigment in the cob: large areas of deep pigmentation often appear in cobs of plants carrying the original state of a_1^{m-2} and an active *Spm* element. They are not due to a heritable modification of a_1^{m-2}, as is shown by the results of tests and is also indicated by the fact that kernels above these areas have the same phenotype as other kernels on the ear. A heritable change would be reflected both in the cob areas and in the kernels overlying them, since the a_1^{m-2} locus in the cells of all is descended from one that was present in a common ancestor cell.

Extension of Spm Control of Gene Action

These studies, over a period of years, have uncovered a number of examples of gene control by foreign systems that have not yet been reported because of inadequate identification. Whether or not the system controlling a particular gene was *Ac* could be determined rapidly, because precise methods of testing were available. Early methods for testing whether or not a system might be *Spm* were much less direct and, as applied to some genes, were considered too indirect to be efficient. Therefore some of the isolates, after they had been found not to represent *Ac* control, were temporarily dropped from study. When it was determined that wx^{m-8} was under the control of the *Spm* system, it became possible to construct tester stocks that could directly reveal *Spm* control of gene action at other loci. Five suspected examples of control of gene action by this system could now be tested with wx^{m-8}. Two, isolated some years ago, involve the C_1 locus in chromosome 9 and are designated c_1^{m-5} and c_1^{m-6}. Two others, recently isolated, involve the C_2 locus in chromosome 4 and are designated c_2^{m-1} and c_2^{m-2}. The last involves the *Pr* locus in chromosome 5.

This past year, the required combinations of gene markers for such tests were available only in plants having c_1^{m-5}. Plants that were c_1^{m-5} Sh_1 wx/c_1 sh_1 wx^{m-8} in constitution were crossed with plants that were homozygous for c_1, sh_1, and wx and carried no *Spm*. Other plants, c_1^{m-5} wx/c_1 wx in constitution, were crossed with plants that were c_1 wx^{m-8}/c_1 wx and had no *Spm*. Among the kernels on the ears produced by these crosses, response of wx^{m-8} to *Spm* was correlated with the presence of c_1^{m-5} in the kernel. These initial tests placed *Spm* close to the locus of c_1^{m-5} in the examined plants. They also indicated that the origin of some of the stable mutants produced by c_1^{m-5} was associated with transposition of *Spm* to a new location in the chromosome complement.

Further confirmation of *Spm* control of gene action at c_1^{m-5} was obtained from tests conducted with one plant in which *Spm* underwent change from the inactive to the active phase late in development. On the ears of this plant, some kernels that had received both c_1^{m-5} and wx^{m-8} gave no evidence of the presence of *Spm* except in one sector, produced by descendants of the cell in which *Spm* had changed to the active phase. A well defined area in the aleurone layer exhibited the types of pigmented spots that are produced by change in gene action at c_1^{m-5}; and only in the underlying cells, derived from the common ancestor cell, did the starch in individual cells or clusters of cells display a phenotype produced by mutation at wx^{m-8}.

It may be added here that wx^{m-8} has also been useful in analyses of change in phase of activity of *Spm* at the a_1^{m-2} locus. Responses of the two loci to phase of activity of *Spm* are similarly correlated.

Further Studies of Topographical Relations of Elements of a Control System

In *Year Book 61*, the origin of a two-element system of control of gene action from an apparently single-element control system was outlined with respect to the *Ac* system in its relation to bz^{m-2} and one of its altered states, Bz^w. Initially,

the regulator element, *Ac*, was present at or very close to the locus of the bronze gene, which is involved in the biosynthetic pathway leading to anthocyanin formation in plant and kernel. In studies, with bz^{m-2}, of the relation between transposition of *Ac* away from the bronze locus and initiation of *Bz* gene action, one instance was found in which *Bz* expression appeared in conjunction with transposition of *Ac* to a position in chromosome 9 between the locus of the *Bz* mutant and that of *Wx*, approximately 10 crossover units distal to *Wx*. In one type of testcross, pollen from a plant that was *I Sh Bz(standard) wx Ds/C sh Bz(mutant) Ac Wx* in constitution was placed on the silks of ears of plants that were homozygous for *c*, *sh*, *Bz*, and *wx* and had no *Ac*. On one of the resulting ears, a single nonpigmented kernel was present in which changes in *Wx* gene action, from a low level to a high level of gene expression, had occurred in some cells during endosperm development.

In order to examine the system responsible for control of *Wx* gene action in this kernel, a plant was grown from it in the summer of 1961. When pollen collected from this plant was stained with an I-KI solution, half the grains stained a red-brown (*wx*) but, unexpectedly, the other half stained a deep blue as if to indicate the presence of a normal *Wx* gene. It was then suspected that only the endosperm of the kernel that gave rise to the plant had received the modified *Wx* gene, and that the normal *Wx* gene had been delivered to the zygote. To verify this suspicion, a few testcrosses were conducted with the plant. It was crossed reciprocally with an *Ac*-tester plant, homozygous for *C*, *Sh*, *Bz*, *wx*, and *Ds* (standard location). It was also crossed to plants that were homozygous for *C*, *sh*, *bz*, and *wx*, either with or without *Ac* in their nuclei, and to a plant whose constitution was *c Sh bz wx/c sh bz wx*, no *Ac*. The kernels produced by these crosses revealed that the zygote had, in fact, received the modified *Wx* gene and that its action was under the control of the *Ac* system. Moreover, *Ac* was found to be located very close to this modified *Wx* gene, which was then designated wx^{m-9} because it represented the ninth detected change in the Cold Spring Harbor cultures whereby the action of the normal *Wx* gene had become subject to a foreign control system.

Results of these initial tests and of others conducted during the past year show that return to a high level of *Wx* gene action is usually associated with removal of *Ac* from the locus of wx^{m-9}. The results of two types of testcross conducted with plants carrying wx^{m-9} are given in tables 1 and 2. They demonstrate

TABLE 1. Phenotypes of Kernels on Ears Produced by Reciprocal Crosses of Plants Having the Constitution $c_1\ wx^{m-9}/c_1\ wx$ with *Ac*-Tester Plants That Were Homozygous for C_1, *wx*, and *Ds* (Standard Location) and Had No *Ac*

♀ = heterozygote employed as ear parent; ♂ = heterozygote employed as pollen parent.

Level of Expression of Waxy Gene in Starch of Endosperm	Pigmentation of Aleurone Layer				Totals
	Uniformly Pigmented (No *Ac*)		Colorless Areas in Pigmented Background (*Ac*)		
	♀	♂	♀	♂	
High level throughout (germinal mutation)	8	11	12	11	42
Low level with sectors of high level	0	0	1834	986	2820
Low level throughout	5	3	0	0	8
Null level (*wx* allele)	1818	1003	30	64	2915
Totals	1831	1017	1876	1061	5785

TABLE 2. Phenotypes of Kernels on Ears Produced by Reciprocal Crosses of Plants Having the Constitution *I* wx^{m-9}/c_1 *wx* with *Ac*-Tester Plants That Were Homozygous for C_1, *wx*, and *Ds* (Standard Location) and Had No *Ac*

The colorless kernels received *I*, whereas the pigmented kernels received the allele c_1 from the heterozygous parent. ♀ = heterozygote employed as ear parent; ♂ = heterozygote employed as pollen parent.

Level of Expression of Waxy Gene in Starch of Endosperm	Pigmentation of Aleurone Layer						Totals
	Colorless		Uniformly Pigmented (No *Ac*)		Colorless Areas in Pigmented Background (*Ac*)		
	♀	♂	♀	♂	♀	♂	
High level throughout (germinal mutation)	17	1	1	2	2	0	23*
Low level with sectors of high level	1212	117	0	0	418	33	1780
Low level throughout	3	0	0	1	1	0	5
Null level (*wx* allele)	479	51	1214	110	27	0	1881
Totals	1711	169	1215	113	448	33	3689

* Six plants derived from these kernels were examined; 4 had no *Ac*, and 2 had *Ac* unlinked to markers in chromosome 9.

the location of *Ac* before transposition, and the relation of transposition of *Ac* to release of *Wx* gene action from control by the *Ac* system.

Among the total of 9474 kernels recorded in tables 1 and 2, 13 kernels with the wx^{m-9} phenotype showed no evidence of the presence of *Ac*. Two of them appeared on ears produced by crosses conducted with the original plant carrying wx^{m-9}. During the past year, plants were grown from these two kernels and each plant was tested for the presence of *Ac* in its nuclei and also for the response of the derivative of wx^{m-9} to introduced *Ac*. The tests showed that neither plant had *Ac*, and that the low level of gene expression characteristic of derivatives of wx^{m-9} remained unchanged as long as *Ac* was absent. When *Ac* was introduced, however, each responded to it. Mutations occurred in individual cells, whose descendent cells exhibited a high level of *Wx* gene action. The time during development when the mutations occurred reflected the dose of *Ac* present in the nuclei of the kernels: the higher the dose of *Ac*, the later the time of occurrence of mutations. The genic marker constitution of the chromosome 9 carrying wx^{m-9} in these two plants indicated that the removal of *Ac* from the immediate vicinity of the original wx^{m-9} locus could not be attributed to crossing over. Thus, wx^{m-9} provides another example of origin of a two-element system of control of gene action from an apparently one-element system.